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Character, Xanthone Content and Antioxidant Properties of Mangosteen Fruit's Hull (*Garcinia mangostana* L.) at Several Fruit Growth Stadia

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ABSTRACT

The objective of this study was to evaluate the characteristics of mangosteen fruit's hull, xanthone content, and antioxidant potential on various stadia of mangosteen fruit development. The experiment was conducted in September 2006 until July 2008 using randomized complete block design, with three replications at maturation stage i.e. 1, 2, 3, and 4 months after anthesis. The parameters being observed were fruit's hull characters, xanthone content, and antioxidant capacity. The results of this study showed that fruit diameter and fruit weight increased until three months after anthesis and then did not change significantly once they entered the process of maturity. Thickness of fruit's hull differed significantly among maturation stage. The thickness of fruit hull was observed at two months and the highest weight was at three months after anthesis. Xanthone content of mangosteen fruit's hull at a month up to four months after anthesis did not differ significantly however capacity of antioxidant differed significantly among fruit ages, the IC50 values increased with the increase of fruit maturation.

Keywords: Antioxidant, fruit growth, fruit's hull, mangosteen, xanthone

ABSTRAK

Penelitian ini bertujuan mempelajari karakter kulit buah manggis dan kadar xanthone, dan potensi antioksidan pada berbagai stadia perkembangan buah manggis. Penelitian dimulai bulan September 2006 hingga Juli 2008. Penelitian menggunakan rancangan kelompok lengkap teracak, dengan 3 ulangan dan 4 perlakuan umur perkembangan buah yaitu 1,2, 3, dan 4 bulan setelah anthesis. Peubah yang diamati adalah karakter kulit buah, kadar derivat xanthones dan kapasitas antioksidan. Hasil penelitian menunjukkan bahwa diameter buah dan bobot buah meningkat hingga buah berumur 3 bulan setelah anthesis dan kemudian tidak berubah ketika memasuki proses akhir pematangan. Tebal kulit buah berbeda antar stadia umur buah, dengan tebal kulit terbesar pada umur 2 bulan setelah anthesis; bobot kulit tertinggi saat umur 3 bulan setelah anthesis. Kandungan xanthone kulit manggis pada umur buah 1 hingga 4 bulan setelah anthesis tidak mengalami perbedaan. Sedangkan kapasitas aktivitas antioksidan kulit buah manggis berbeda antar umur buah, nilai IC50 mengalami peningkatan sesuai umur buah.

Kata kunci: manggis, perkembangan buah, kulit buah, xanthones, antioksidan

INTRODUCTION

Besides as a fresh fruit, mangosteen is also used as natural medicine, especially from its hull by people in some Asian countries including Indonesia. Increasing opportunities in the value of the mangosteen fruit more prospective with the discovery of the xanthone content of bioactive compounds in some parts of the mangosteen plant. Mangosteen xanthone biosynthesis in plants is very poorly investigated, as well as environmental factors that affect biosynthesis.

The results of the xanthone which has been widely reported is limited to the isolation and identification of structures (Chairungsrilerd *et al*., 1996; Komguem *et al*., 2005; Tanaka and Takashi, 2006) and efficacy (Chin *et al*., 2008; Han *et al*., 2008). Xanthone pharmacological activity has been reported such as an antibacterial (Suksamrarn *et al*., 2003; Komguen *et al*., 2005), anticancer (Hong *et al*., 2004) and antioxidant (Moongkarndi *et al*., 2004; Lannang *et al*., 2005; Mahabusarakam *et al*., 2005; Mahabusarakam *et al*., 2006).

Mangosteen fruit hull is one part that has potential to be utilized as raw material for phytopharmaca or natural compounds source. But until now there is no information about the content of xanthone in mangosteen fruit hull when the fruit is in the process of development. Chemical character of the mangosteen fruit changes during process of growth and fruit development. During the fruit ripening process physico-chemical properties will change, which

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generally consists of changing of colors, the composition of the cell wall (texture), starch, protein, phenol derivative compounds and organic acids. Fresh fruits after harvesting are still having the biological processes (Winarno and Wirakartakusumah, 1981). Changes in chemical and biochemical persist because tissues and cells still showed metabolic activity (Eskin, 1990).

Research to study the xanthone accumulation during fruit development in mangosteen has not been performed. Nevertheless, such research is important and necessary for the development of mangosteen as a producer of natural compounds. The results of these studies are expected to provide benefits for developing phytopharmaca mangosteen-based fruit hull.

This research aims studying the development of the mangosteen fruit, xanthone accumulation in the fruit hull, and antioxidant properties of the various stadia development of the mangosteen fruit hull.

MATERIALS AND METODS

The study was conducted September 2006 through July 2008. Fruit sampling conducted in the mangosteen production center at Cengal, Karacak Village, Leuwiliang District, Bogor Regency. Observations of fruit morphology and extraction of fruit hull conducted at the Eco-physiology Laboratory, Agronomy and Horticulture Department, content analysis of xanthones derivate at Integrated Laboratory, Agriculture Faculty, Bogor Agriculture University (IPB); antioxidant properties assay at the RGCI laboratory, Agronomy and Horticulture Department, IPB.

Plant material used was mangosteen tree about 30 years old, has been producing, healthy and flowering. Other materials used are methanol (Merck) and xanthone (Sigma). The tools used were analytical scales, penetrometer, digital caliper, blades, evaporator, spektrophotometer UV-VIS, and HPLC.

The experiment used randomized block design with a single factor and four levels of fruit growth stage i.e 1, 2, 3 and 4 month after anthesis (MAA). The treatment consisted three replication and 3 trees per treatment. Twenty fruit were taken for each treatment in every tree.

The experiment began with determining the mangosteen tree ± 30 years old, has been productive, healthy, followed by labelling of flowers on anthesis. Mangosteen fruits were harvested in accordance with treatment. Observations parameters were fruit diameter, total weight, weight of hull, aril + seed weight, skin thickness, score of yellow sap and scab following Kartika (2004), content of xanthones and benzophenone, and antioxidant properties measured as the ability of the capture of free radicals by the method of DPPH (2.2-*diphenyl-1-pikrilhidrazil*).

Content of xanthones and benzophenone were measured using following procedures. Extraction was conducted from dried mangosteen's hull in the form of powder, i.e. as much as 100 g powder was extracted with methanol solution of 100 mL (p.a). Analysis of benzophenone and xanthones was carried out by using methanol eluent and formic acid; and was detected at wave length of 234 nm (Teixiera *et al*., 2003).

The activity of free radical scavengers was measured using DPPH method. As much as 1 mL extract in various test concentrations were added 1 mL of DPPH 0.4 mM and 3.9 mL of ethanol were added into 100 µL of extract in various test concentrations. The absorbance was measured using spectrophotometer UV-VIS (Rohman and Riyanto, 2005). The antioxidant potential were presented in the form of IC_{50}.

Data were analyzed using ANOVA (F Test) at level α = 5% and further test was carried out with Duncan multiple range test at level α = 5%.

RESULT AND DISCUSION

Fruit Character

Fruit growth stadia influenced fruit weight and fruit diameter significantly. Fruits that were at 3 and 4 MAA growth stage were heavier and bigger than those that were 1 and 2 MAA growth stage (Table 1). Aril and seed weight continued to increase as the fruit age increase until the fruit ripening period, while the weight of the hull decreased when the process of ripening fruit begins (Table 2). This is presumably because the cellulose and hemicellulose in the hull during fruit ripening period is converted to starch (Simmond, 1966). The size of fruit produced is determined by the sink strength during fruit growth process, whereas the sink strength is determined by physiological conditions

Table 1. Physical fruit character at various fruit growth stage

Fruit stage (MAA)	Fruit weight (g)	Fruit diameter (mm)	Weight of aril+seed	Weight	Scab score	Yellow sap score
1	16.50c	27.63c	0.89d	3.80	2.3	2.7b
2	46.28b	43.25b	5.75c	3.75	3.0	3.0ab
3	75.51a	52.02a	19.09b	4.94	3.0	3.7a
4	74.99a	51.80a	23.77a	3.41	2.3	3.0ab
F test	**	**	**	ns	ns	*

Note: Number followed by the same letter at the same column did not differ significantly by DMRT at α = 5%; * = significant at α = 5%; ns = not significant; MAA = Month After Anthesis from synthesis of asimilate to accumulation in the fruit (Yamaki, 2010). If is thought that at the age of 1-2 month, ‘sink’ is formed strongly, therefore the process of fruit development is determined in this period.

The intensity of the scab did not differ among fruit ages. It means that the scab occurs since the initial fruit growth up to harvest with the same intensity. The scab is probably caused by friction between fruit or fruit with leaves when the fruit is young and leaves wounds that come enlarge so the hull color becomes dull, rough surface and affect the external appearance of the mangosteen.

The intensity of the yellow sap on fruit hull increased until 3 MAA and then decreased during maturation. Symptoms of yellow sap is a major problem in mangosteen. Until now, there are several theories about yellow sap, the sap of which is yellow resin exudate encountered various plants of the family Guttiferae, resin derived from the damaged channels (Asano *et al*., 1996; Pankamsemsuk *et al*., 1996). Humid conditions of the planting area which is favourable for *Fusarium oxysporum* attacks on fruit could induce the yellow sap (Verheij, 1997). The emergence of yellow sap occur because of damage to secretory channel so that the sap out of yellow gum littering the mangosteen aril (Poerwanto, 2010). Parts of mangosteen fruit that does not change from early growth to harvest is the fruit stalk.

Fruit Hull Character

Thickness of fruit hull was significantly different among the fruit stage. Fruit with the greatest thickness was fruit at 2 MAA (Table 2). Mangosteen fruit hull thickness increased significantly from age 1-2 MAA, and further decreasing until the fruit ready to harvest. This condition is contrary to the development of mangosteen fruit and seeds, where growth of the fruit flesh and seeds of fruits increased with age. The development of mangosteen hull weight increased with age. When the fruit entered the maturation period, i.e. the age of 3 MAA, there was a decrease in hull thickness and weight. Thus, fruits at 4 MAA had the thinnest (0.69 cm) hull (Table 2). However, the weight of fruit hull at 4 MAA did not differ than those at 3 MAA.

At the early fruit growth, i.e. until 4 MAA, the fruit hull of mangosteen is the largest portion of the mangosteen fruit. The portion of fruit hull to weight fruit is increased until 2 MAA and reached 80%. However, the portion of fruit hull to weight fruit is decreased until fruit ripening i.e. 68% fruit hull. Gunawan (2007) and Sidik (2004) reported that the edible portion of the mangosteen fruit is only 35.07%. The highest potential of biomass production of fruit hull comes from fruit at 3-4 MAA stage.

Content of Xanthone Derivates

Xanthones content did not change from young fruit until 4 MAA, about 14,670-16,206 $\mu g\ g^{-1}$ crude extract of mangosteen hull (Table 3). The levels of benzophenone, which is intermediate in the formation of xanthone compounds did not change. D’iaz-Mula (2008) reported that the major changes associated with ripening fruit are changes in color, total soluble solid, total titratable acid, and content of bioactive (anthocyanin and carotenoids). Similarly, the report of Awad *et al*. (2001) showed that there is a change in bioactive content during fruit development; anthocyanin content of apple skin in the early growth is relatively high and gradually decreases until it reaches a fixed point during fruit growth and then begins to increase when the stage of fruit ripening.

The xanthone content of fruit hull is relatively constant during fruit growth. This is more profitable because fruit hull from various fruit stage can be use as xanthone source, not limited to ripe fruit only but also from immature fruits. Kartika (2004) showed that in Leuwiliang fruitset percentage was 91.14%, but most of them (41%) fall in immature stage. Furthermore, based on the results of this study xanthones synthesized from 1 MAA could reach 14,670 μg^{-1} of crude extract of mangosteen hull. Thus, fallen immature fruits can be used as a source of xanthone derivates.

Antioxidant Properties

IC_{50} values increased with fruit growth (Table 4). The highest antioxidant potential are from fruit with the age

Table 2. Physical character of mangosteen fruit hull at various growth stage

Fruit stage (MAA)	Hull thick (cm)	Fresh weight of fruit hull (g)
1	0.82bc	11.82c
2	1.01a	36.79b
3	0.93ab	51.48a
4	0.69c	47.81ab
F test	**	**

Note: Number followed by the same letter at the same column did not differ significantly by DMRT at α = 5%; * = significant at α = 5%; ns= not significant

Table 3. Content of xanthone derivates at fruit hull extract of various fruit growth stage

Fruit stage (MAA)	Xanthone derivate	Benzophenone
	($\mu g\ g^{-1}$)	
1	14,670	8,483
2	16,206	7,936
3	15,741	8,308
4	15,680	10,795
F test	tn	tn

Note: Number followed by the same letter at the same column did not differ significantly by DMRT at α = 5%; * = significant at α = 5%; ns= not significant of 1-2 MAA, i.e. about 6.31-6.80 ppm. It means that the ability to capture free radicals is higher when the fruit was young and its activity decrease as the fruit age increase. This is apparently associated with decreased synthesis of antioxidants during the mangosteen fruit ripening process resulting in oxidative stress. As a reported by Franklin *et al*. (2009) in cell culture *Hypericum perforatum* that is act as aphytoalexin, xanthones acts as an antioxidant to protect cells from oxidative damage. Celik *et al*. (2008) reported that the chemical character and antioxidant capacity of fruit influenced by fruit growth stage, immature fruit contains the highest antioxidant capacity. Huang *et al*. (2007) also reported that the decline of non-enzyme antioxidants in the late phase of fruit enlargement associated with decreased antioxidant activity thereby increasing oxidative stress and lead to metabolic changes associated with fruit ripening and aging of citrus fruits.

Table 4 . Antioxidant properties of the fruit hull extract at various fruit growth stage

Fruit stage (MAA)	IC_{50} DPPH (ppm)
1	6.31c
2	6.80c
3	9.57b
4	12.81a
F test	**

Note: Number followed by the same letter at the same column did not differ significantly by DMRT at α = 5%; * = significant at α = 5%; ns= not significant

CONCLUSION

Fruit diameter and fruit weight increased until fruit age 3 MAA and then did not change significantly when entering the final process of maturation. Hull thickness differed significantly among fruit growth stage, the highest thickness was from 2 MAA. Fruit hull weight increased as fruit growth stage increase with the highest weight at 3 MAA. Xanthones content of mangosteen fruit hull from 1-4 MAA did not differ, while the capacity to capture free radical was significantly different between the age of the fruit. The highest antioxidant potential are from fruit with the age of 1-2 MAA, and decreased as the fruit age increase.

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